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PRINCIPAL INVESTIGATOR: Janani Krishnamurthy

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Krishnamurthy, Janani

E-Mail: JKrishnamurthy@mdanderson.org

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14. ABSTRACT

Human endogenous retroviruses (HERVs) are ancient viruses forming 8% of human genome. One subset of HERVs, the HERV-K has recently been found to be expressed on tumor cells not on normal body cells. Thus, targeting HERV-K protein as a tumor associated antigen (TAA) may be a potential treatment strategy for tumors that are resistant to conventional therapies. Recognition of cell-surface TAAs independent of major histocompatibility complex can be achieved by introducing a chimeric antigen receptor (CAR) on T cells using gene therapy. Preliminary analysis of HERV-K env protein expression in 268 melanoma samples and 139 normal organ donor tissues using immunohistochemistry demonstrated antigen expression in tumor cells and absence of expression in normal organ tissues. *The scFv* region from a mouse monoclonal antibody to target *HERV-K* env was used to generate a CAR and cloned into Sleeping Beauty (SB) plasmid for stable expression in T cells. HERV-K-specific CAR$^+$T cells were selectively propagated *ex vivo* on artificial antigen presenting cells (aAPC) using an approach already in our clinical trials. 95% of propagated T cells stably expressed the introduced HERV-K-specific CAR and exhibited redirected specificity for HERV-K$^+$ melanoma. Further, the adoptive transfer of HERV-K-specific CAR$^+$T cells killed metastatic melanoma in a mouse xenograph model. These data demonstrate that it is feasible to generate T cells expressing a HERV-K-specific CAR using a clinically-appealing approach as a treatment strategy for HERV-K env$^+$ tumors.

15. SUBJECT TERMS
Chimeric antigen receptor, T cell therapy, Human endogenous retrovirus, cancer

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Introduction:
During the human genome project, a set of ancient retrovirus named Human endogenous retrovirus (HERVs) was discovered to be stably integrated into the human genome forming 8.5% of the total human genome[1]. Among the HERVs, HERV-K was found as an oncogenic allelic variant involved in melanoma, breast cancer, ovarian cancer, tretatocarcinoma and prostate cancer along with various autoimmune diseases like multiple sclerosis and rheumatoid arthritis[2-4]. The oncogenic potential of HERV-K is contributed by the envelope (env) and GAG protein (Rec). Recent studies have shown that the expression of the HERV-K env protein exclusively on tumor cell surface and not on normal skin cells[5,6]. The expression of HERVK env protein was found to increase with more aggressive and metastatic type III and type IV melanoma than less aggressive and localized type I melanoma[2,7,8]. This selective expression of HERV-K env protein on melanoma cells can be harnessed as a treatment strategy for patients with refractory or metastatic melanoma. Patients with metastatic melanoma have a poor prognosis due to resistance to conventional therapies such as chemotherapy, radiation and surgery[9]. Thus, new targeted treatment strategies are required to improve therapeutic outcome.

One prospective target-specific treatment strategy which will increase the prognosis of the patient with aggressive metastatic melanoma is immunotherapy. Adoptive T cell therapy is a target-specific immunotherapy involving T cells infusions which are rendered specific for tumor-associated antigens (TAAs) preferentially expressed on melanoma cells[10,11]. Recognition of cell-surface TAAs independent of major histocompatibility complex can be achieved by introducing a TAA-specific chimeric antigen receptor (CAR) on T cells using gene therapy. The CAR is introduced into the T cells using a non-viral Sleeping Beauty (SB) transposon/transposase system. These CAR^+T cells are then grown in antigen selective manner using artificial antigen presenting K562 cells (aAPCs). The antigen specific T cells can localize in the antigen rich tumor site and ablate the melanoma cells by T-cell mediated killing which includes releasing of perforin, granzyme-B and interferon-γ[10].
Purpose: Adoptive T cell therapy is a novel immunotherapy technique in which T cells can be genetically modified to render them specific for cell surface molecules such as HERV-K by introducing a chimeric antigenic receptor (CAR)[11]. These CAR's are typically formed by fusing the scFv sequence of monoclonal antibody (mAb) specific for the target antigen to Fc stalk and a CD28 and CD3ζ sub-domains for fully competent intracellular signaling[12]. Clinical grade T cells can then be grown in the presence of irradiated artificial Antigen Presenting Cells (aAPCs) which endogenously express HERV-K antigen leading to selective propagation of HERV-K CAR^+ T cells. These CAR^+ T cells can now be specifically targeted against HERV-K^+ melanoma tumor.
Scope: This work has tremendous translational implications in the field of cancer. Here we understand the nature of virus activity in the cancer cell and its involvement in cancer prognosis. Melanoma forms 80% of all skin cancer and about 10% of all metastatic melanoma cancer patients do not live longer than 10 years[9]. Here we have a new treatment strategy where we determine the ability of HERV-K CAR^+ T cells to preferentially target HERV-K antigen positive melanoma cells *in vitro* and *in vivo*. This approach is based on our clinical trial infusion using genetically modified T cells expressing a CD19-specific CAR in patients with B-lineage malignancies[11].

Body: The HERV-K specific CAR is electroporated in PBMCs from normal donors and expanded in an antigen specific manner. These HERV-K CAR^+ T cells are its specificity and tumor killing ability *in vivo* and *in vivo*.
Aim 1: To develop HERV-K-specific CAR-$ffLuc^+$T cells by *Sleeping Beauty* (SB) mediated transposition (0-10 months):
Preparation of HERV-K-specific CAR: HERV-K antigen specific monoclonal antibody (mAb) was developed in mouse and the 6H5 mAb clone was found most sensitive in detecting antigen *in vitro*[1]. The scFv sequence of the 6H5 mAb clone was used to construct the CAR. The scFv cassette was fused to

IgG4Fc region by a flexible linker, followed by CD28 transmembrane and CD28 and CD3ζ intracellular domain. This was then cloned in SB transposon vector (Figure 1A).

Expansion of HERV-K-specific CAR$^+$ T cells: To generate CAR$^+$ T cells specific to HERV-K antigen on T-cell surface, transposition of HERV-K CAR transposon along with the SB11 transposase vector to facilitate the integration of the gene was performed. PBMCs electroporated with the two vectors were then propagated on K562 derived aAPC in the presence of IL-2 and IL-21. K562 aAPCs, endogenously expressing the HERV-K antigen, were genetically modified to co-express desired T-cell co-stimulatory molecules CD86, 4−1BBL, and membrane bound IL-15 (co-expressed with enhanced green fluorescent protein, EGFP)[11,12]. Irradiated aAPCs were supplemented every 7 days to stimulate the growth of HERV-K-specific CAR$^+$ T cells. PBMCs without any transposon electroporated, grown on OKT3 loaded aAPCs under the same culture conditions served as a negative control.

Expression and expansion kinetics of HERV-K-specific CAR$^+$T cells: The expression of CAR was detected using a polyclonal Fc antibody specific for the IgG4Fc region of the HERV-K CAR. The CAR$^+$ T cells were stained with the Fc antibody every week before the supplementing the culture with irradiated aAPCs. Flow data revealed that 95% of T cells express CAR on its surface after 35 days of co-culture with aAPC (Figure 1B).
No significant difference in growth kinetics of total HERV-K CAR$^+$ T cells was seen when compared to No DNA T cells and most of the CAR$^+$ T cells were CD3$^+$ T cells by Day 20 of culture (Figure 1C and D). The percentage of CAR$^+$ CD4 and CAR$^+$ CD8 cells increases along the culture period and most of these CAR+T cells have an effector memory phenotype (Figure 1E). These data suggest that the T cells electroporated with HERV-K had stable expression of the CAR.

Efficacy and specificity of HERV-K-specific CAR$^+$T cells: The antigen expression on tumor cells surface is shown in Figure 2A. Chromium release assay (CRA) was performed and the antigen specific HERV-K$^+$ tumor lyses was analyzed after a period of 4hrs co-culture with HERV-K-specific CAR$^+$ T cells and compared with HERV-K^{neg} target cells. A graded killing was observed between the different ratios CAR$^+$ T cells to fixed ratio of target cells. An increase in target cell lysis was observed with increased HERV-K antigen expression when compared to EL4 parental cell as negative control (Figure 2B). No significant killing was observed with No DNA control T cells with the HERV-K$^+$ and HERV-K^{neg} tumor targets (Figure 2B). The data represents average of triplicates from three different donor derived HERV-K CAR$^+$ T cells.
The functionality of HERV-K CAR$^+$ T cells was determined by measuring IFN-γ production after four-hour co-culture with the melanoma tumor cell lines at a 1:5 (T cell: Tumor cell) ratio. Significant increase in IFN-γ production in CAR$^+$ T cells co-cultured with HERV-K$^+$ targets compared to tumor cells co-cultured with No DNA control T cells. Absence of such difference was seen with EL4 parental target cells. PMA- ionomycin was used as a positive control while no target was used as negative control (Figure2C).These data demonstrate that HERV-K CAR$^+$T cells can be specifically redirected against HERV-K antigen expressing tumor.
To analyze the specificity of HERV-K CAR, EL4 cells (HERV-K ^{neg}) were transduced with bi-directional SB plasmid encoding HERV-K envelope protein and neomycin drug selection gene along with SB11 transposase (Figure 2D). Single cell clones of HERV-K$^+$ EL-4 cells were used to determine the re-directed specificity of HERV-K CAR$^+$ T cells. HERV-K$^+$ EL-4 cells had significantly increased cell lysis in the presence of varying ratios of HERV-K T cells when compared to EL-4 parental cells which are negative for antigen expression (Figure 2E). The data represents an average of 3 readings from three donor derived HERV-K CAR$^+$ T cells. SiRNA was sued to knock down HERV-K antigen in A88 mel cells and produced about 40% reduction in antigen expression (Figure 2F). These cells

exhibited reduced killing by HERV-K-specific CAR+T cells compared to A888 parental or the treated scrambelled control siRNA (Figure 2G)

Comparison of HERV-K-specific CAR$^+$ T cells and HERV-K-specific CAR-ffLuc$^+$ T cells: PBMCs electroporated with HERV-K-specific CAR and myc-ffLuc plasmids and grown on K562 aAPCs in the presence of IL-2 and IL-21. The growth kinetics, cytotoxicity and specificity of HERV-K-specific CAR-ffLuc+T cells and HERV-K-specific-CAR+T cells were not significantly different (p-value >0.05) (Supplementary Figure 1A-C)

Aim 2: To assess the mb-IL-12 mediated CAR-ffLuc$^+$T cell activation in an immunesupressive tumor environment (0-12 months):

Generation of mb-IL-12 plasmids: To generate the mb-IL-12 onto T cell surface, 4 different mb-IL-12 fusion sequences was used. This included sequence using either GM-CS for IL-12 signal peptide or Fc-CD4 or Fc- CD8 stalk (Figure 3A). All the 4 sequences generated were cloned in SB bi-directional plasmids which encode for Neomycin resistance gene under cytomegalovirus (CMV) promoter and mb-IL-12 under human elongation factor-1α (hEF-1a) promoter (Figure 3B). The mb-IL-12 with the Fc-CD8 stalk was cloned into a unidirectional SB plasmid under NFAT promoter with a P2A linker followed by neomycin resistance gene (Figure 3C).

Electroporation and expansion of mb-IL-12$^+$ HERV-K CAR$^+$ T cells in an antigen specific manner: To generate mb-IL-12$^+$CAR$^+$ T cells specific to HERV-K antigen on T-cell surface, double transposition of HERV-K CAR transposon and mb-IL-12 SB plasmid along with the SB11 transposase vector to facilitate the integration of the gene was performed (Figure 4). PBMCs electroporated with the three plasmids were then propagated on K562 derived aAPC in the presence of IL-2 and IL-21. K562 aAPCs, endogenously expressing the HERV-K antigen, were genetically modified to co-express desired T-cell co-stimulatory molecules CD86, 4−1BBL, and membrane bound IL-15 (co-expressed with enhanced green fluorescent protein, EGFP)[7, 8]. Irradiated aAPCs were supplemented every 7 days to stimulate the growth of HERV-K-specific CAR$^+$ T cells. 0.8mg/ml of G418 neomycin was also supplemented to select CAR$^+$T cells expressing mb-IL-12. PBMCs without any transposon electroporated, grown on OKT3 loaded aAPCs under the same culture conditions served as a negative control. CAR$^+$ T cells grown in the presence of soluble IL-12 was used as a positive control.

Expression and expansion kinetics of mb-IL-12$^+$ HERV-K CAR$^+$T cells: The expression of CAR was detected using a polyclonal Fc antibody specific for the IgG4Fc region of the HERV-K CAR. The expression of IL-12 was detected using monoclonal antibody against the p40 and p35 region of IL-12. No significant difference in growth kinetics of total HERV-K CAR$^+$ T cells was seen when compared to No DNA T cells and most of the CAR$^+$ T cells were CD3$^+$ T cells by Day 20 of culture (Figure 5A). These data suggest that the T cells electroporated with HERV-K had stable expression of the CAR. The CAR$^+$ T cells were stained with the Fc antibody and for IL-12 every week before the supplementing the culture with irradiated aAPCs. Flow data revealed that 95% of T cells express CAR on its surface after 35 days of co-culture with aAPC (Figure 5B).The expression of IL-12 was restricted inside the cells and was never expressed on the surface. Both p40 and p35 staining data revealed similar results with absence of surface stain. This data was seen with all the above mentioned plasmids of IL-12 including the one with the NFAT promoter (Figure 5C and D).
Chromium release assay and T-reg suppression assay did not yield any significant result due to the absence of surface expression of IL-12 (data not shown).

Aim 3: To determine the anti-tumor effect of mb-IL-12$^+$CAR-ffluc$^+$T cells *in vivo* (0-14 months):
Preparation of Tumor cells and HERV-K-specific T cells for infusion: A375 SM cells obtained from MDACC cytogenetic core facility was transduced with Rluc 8.6535 T2A mKate 5158A (Figure 6A) (renilla luciferase) lenti particles. These cells were sorted for pure mkate expressing population and passaged (intra venous administration) through mice for a period of 2 months to procure the metastatic potential and harvested from the lungs and sorted again for pure tumor population.

Analyzing in vivo tumor ablating efficacy of HERV-K-specific CAR-ffLuc$^+$T cells: Metastatic melanoma model A375-SM transduced mKate-rRluc lentiparticles was infused in 5wk old female NOD/SCID/*IL-2Rγ*(NSG) mouse[13]. HERV-K CAR$^+$ T cells were adoptively transferred into immunocompromised NSG mice bearing established A375-SM metastatic melanoma. Five days following tumor cell engraftment, 20 million HERV-K-specific CAR-ffLuc+T cells were infused intravenously on days 5, 13 and 20 days along with three doses of 600U of IL-2 (*i.p.*) a week for three weeks (Figure 6B). Bioluminescence imaging (BLI) was used to assess the rRLuc activity in each group of mice using enduring as substrate. Mouse group with tumor cells alone had significantly higher renilla luciferase flux by day 25 compared to group having tumor cells and receiving the HERV-K CAR-ffLuc$^+$ T cells (Figure 6C and D). Also mouse with tumor alone became moribund with ruffled fur, unsteady gait, weight loss and hunched posture by day 28 due to the high metastasis of tumor to liver while mouse group receiving the CAR$^+$T cells exhibited healthy appetite and activity (Figure 6E). When assessed upto day 30 was a significant increase in the survival of the treatment group mice compared to no treatment tumor bearing mice (Figure 6F). The liver and lung tissue tumor group mice and tumor with CAR$^+$T cells group imaged for m-Kate expressed on A375-SM cells using a Leica whole body immunofluorescence microscope. The lungs of the tumor alone mice were shriveled and pale compared to treatment mice and there was a significant difference in the number of tumor metastatic colonies between the treatment and non-treatment group suggesting the role of HERV-K CAR$^+$T cells in reducing tumor growth and metastasis (Figure 6G).

Key research accomplishment:
We successfully expanded HERV-K CAR-ffLuc$^+$ T cell population in the presence of aAPCs. The HERV-K-specific CAR$^+$ T cells killed HERV-K$^+$ tumor targets in an antigen specific manner releasing significantly higher amount of IFN-γ. The HERV-K-specific CAR$^+$ T cells were proficient in killing EL4 cells with forced HERV-K antigen expression displaying specificity. Knocking down HERV-K antigen from A888 cells reduced its killing. HERV-K-specific CAR$^+$ T cells were functional in ablating tumor metastasis and growth in mice. Increased survivability of mice with tumor receiving CAR$^+$T cells when compared to mouse with tumor alone was observed.
Successfully designed and made mb-IL-12 plasmids with NFAT promoter. The expression of mb-IL-12 on T cell surface was detected intracellular.
In this report we describe the generation of HERV-K-specific CAR$^+$ T cells, antigen-specific killing of melanoma lines by CAR$^+$ T cells *in vitro* and *in vivo*. These data demonstrates HERV-K CAR$^+$ T cells as a potential therapeutic strategy for patients with aggressive, non-responsive and metastatic melanoma.

Reportable outcomes:
1. Data from this project was presented at the American society of Hematology, Dec 2013 and Immunotherapy congress, May 2013.
2. Metastatic melanoma model was established and HERV-K-specific tumor clearance was detected *in vivo.*
3. Functionality and specificity of HERV-K-specific CAR-ffLuc$^+$T cells *in vivo* and *in vitro* were demonstrated.

Conclusion:
Advanced and relapsed melanoma is generally considered difficult to treat due to development of tumor resistance to conventional therapies. One way to extend patient survival will be to improve the host immune response against their tumor. To accomplish this, we have developed a strategy to infuse large numbers of tumor-specific T cells. Tumor-specificity is achieved by modifying normal donor-derived T cells to express a receptor specific for a novel tumor molecule, human endogenous retrovirus-K (HERV-K). This unique viral antigen was found integrated into the human genome and expressed selectively on melanoma cells and not on normal cells. For tumor-targeting, CAR specific for HERV-K envelope protein was genetically cloned into the T-cell surface using a non-viral vector to integrate CAR into the T cells. These CAR^+ T cells were grown in an antigen specific manner to large numbers using our clinical grade artificial antigen presenting cells (aAPCs) which endogenously express HERV-K. These CAR^+T cells were found to target and reduce the tumor load significantly both *in vitro* and *in vivo* mouse model in an HERV-K dependent manner. Thus, this therapy will benefit patients with aggressive and metastatic melanoma expressing HERV-K antigen. While we have chosen melanoma as our tumor model, this study has the potential to be applied to other malignancies, including breast cancer, lymphoma and myeloma due to restricted expression of HERV-K envelope (env) protein on these tumor cells. Benefits of this therapy include minimized off-target effects since normal tissue does not express HERV-K antigen with successful tumor clearance. This therapy can reduce the need for intense chemotherapeutic regimens and concomitant toxicities. **Future research:** To understand the HERV-K virus as an antigen, structure of viral env protein on the cell surface and the nature of its signal peptide is being analyzed using molecular cloning and mass spectrometry. The virus is known to be shed from the cell surface, hence tagging the virus with RFP and following its activity is under progress to better understand viral activity.

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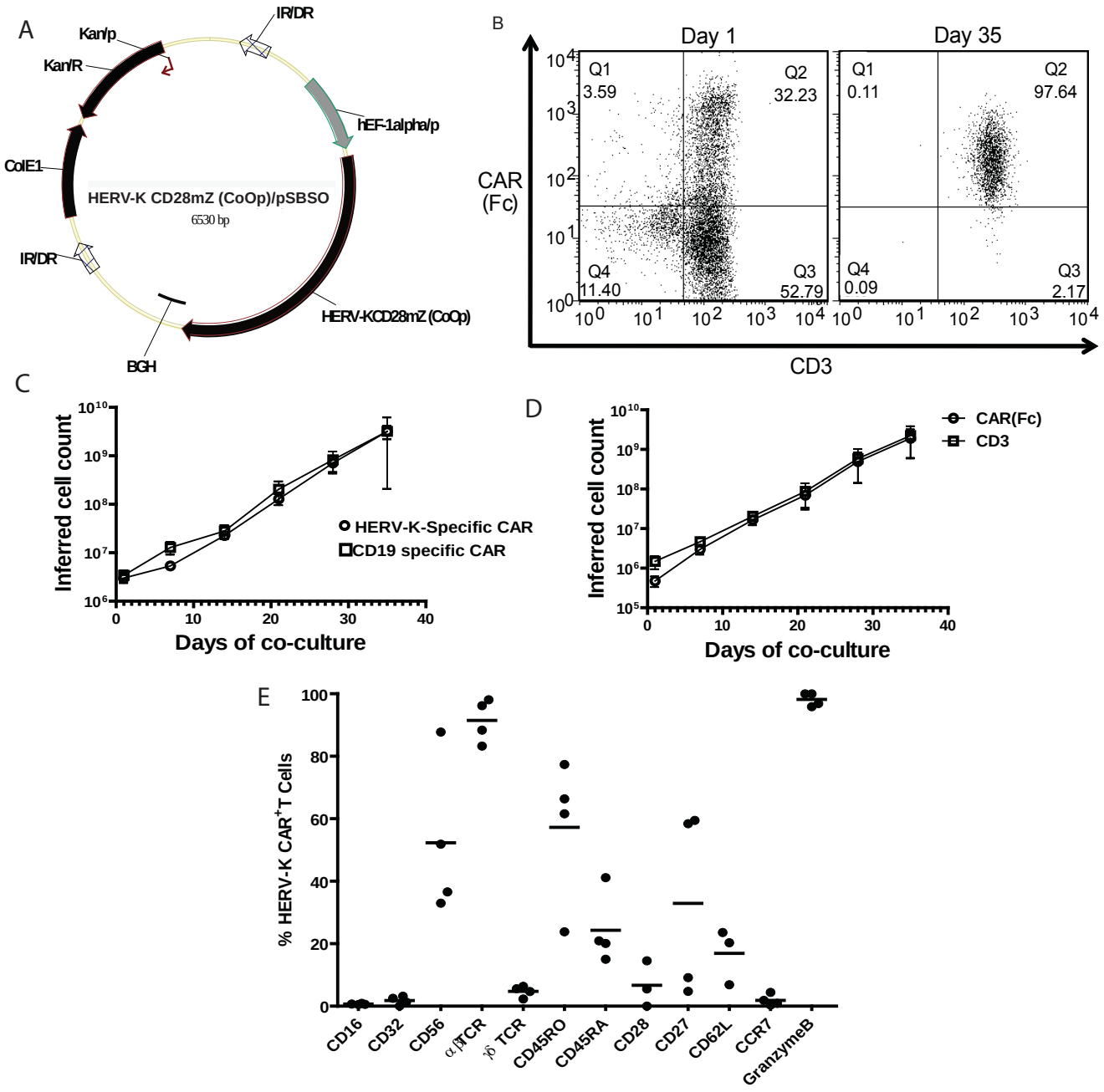


Figure 1: (A) HERV-K-specific CAR encoding SB plasmid. (B) Flow plot representing CAR (Fc) expression on Day 1 and Day 35 of CD3+ HERV-K-specific CAR+T cells. Quadrant percentages of flow plots are in upper right corner. CAR, chimeric antigen receptor. (C) No significant difference in total cell growth between the HERV-K-specific CAR+T cells and non specific CD-19 CAR+ T cells. (D) By Day 21 all HERV-K-specific CAR cells are CD3+T cells. (E) The phenotype of HERV-K-specific CAR+T cells are CD3+CD56+CD45ROhi CD45RAloCD27+CD62L+ T cells that produces high levels of granzymeB. All Data represents average of 4 donors

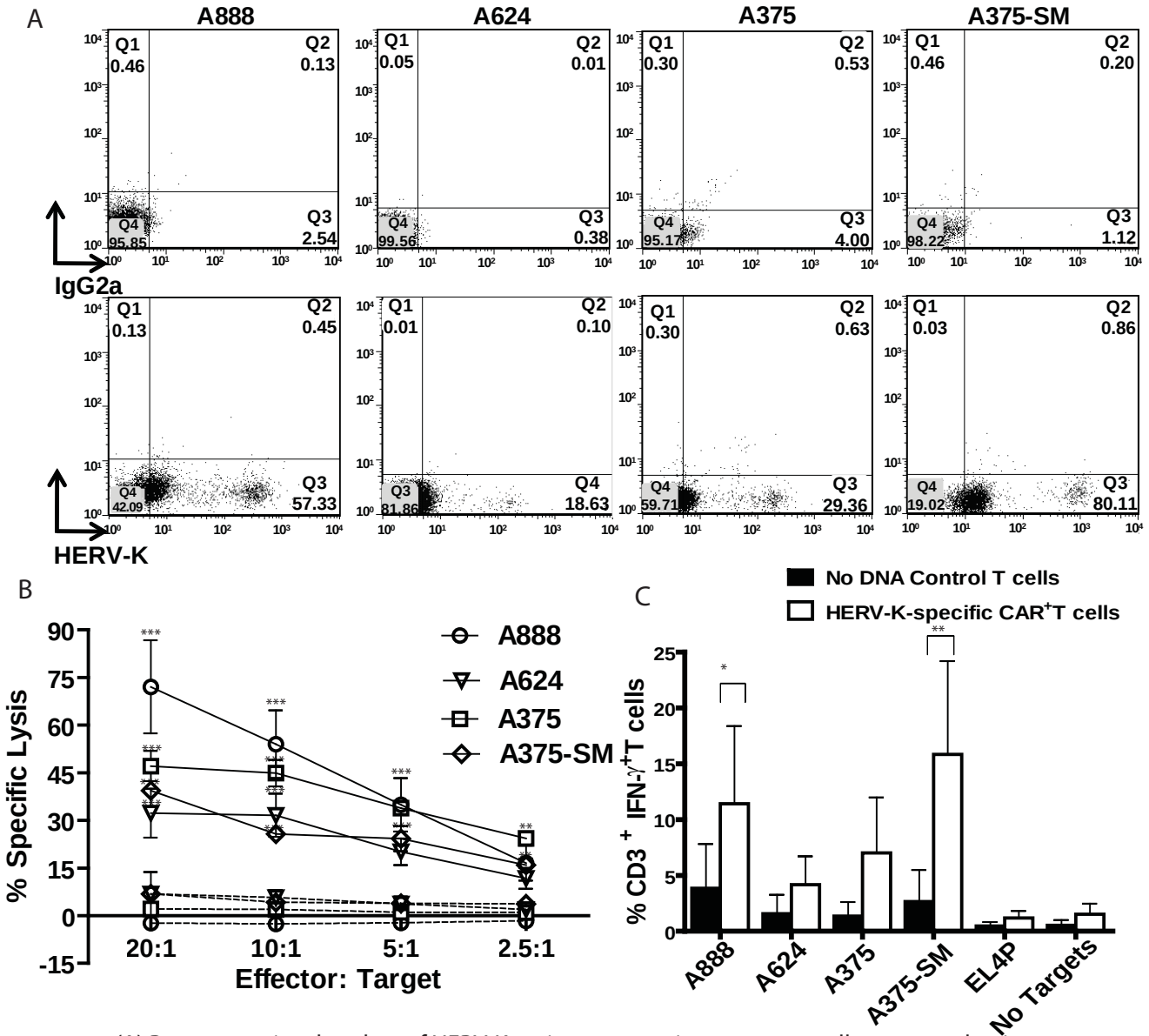


Figure 2: (A) Representative dot plots of HERV-K antigen expression on tumor cells compared to isotype contro (B) A standard 4-hr CRA of melanoma tumor targets with varying dilution of HERV-K-specific CAR+T cells (solid line) compared to No DNA control T cells (dotted lines) (C) IFN-γ production by CAR+T cells upon incubation with targets for 4hrs shows significant difference in production of IFN-g with A888 and A375-SM targets between the No DNA control T cell and the HERV-K-specific CAR+T cells (p-value= 0.02). All data represents mean ± SD from three healthy donors (average of triplicate measurements for each donor) that were pooled from two independent experiments. Two way ANOVA with Bonferroni post-test was performed on (B) and (C) between the HERV-K-specific CAR+T cells and No DNA control cells.CAR, chimeric antigen receptor; CRA, chromium release assay; E:T, effector to target ratio

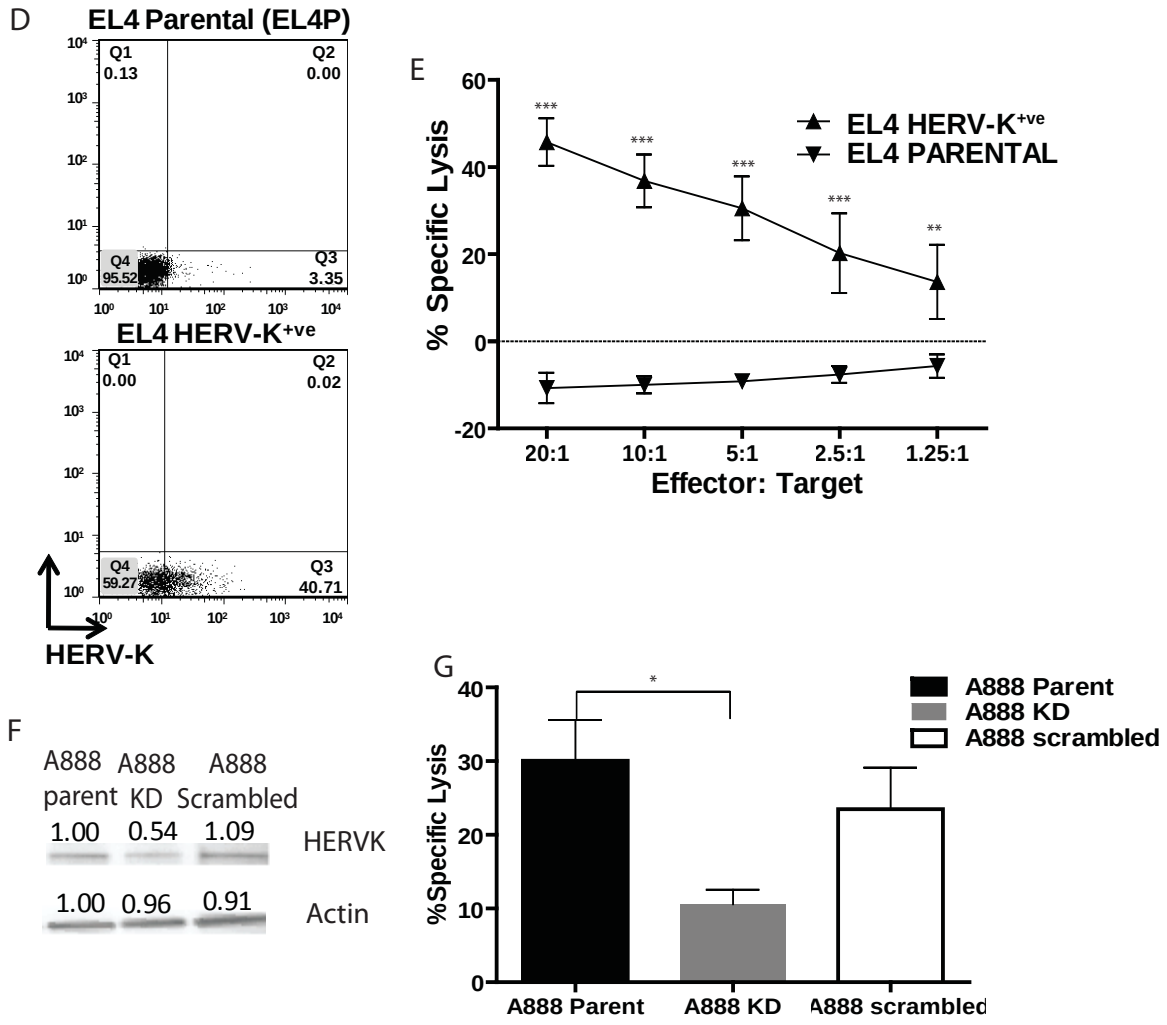
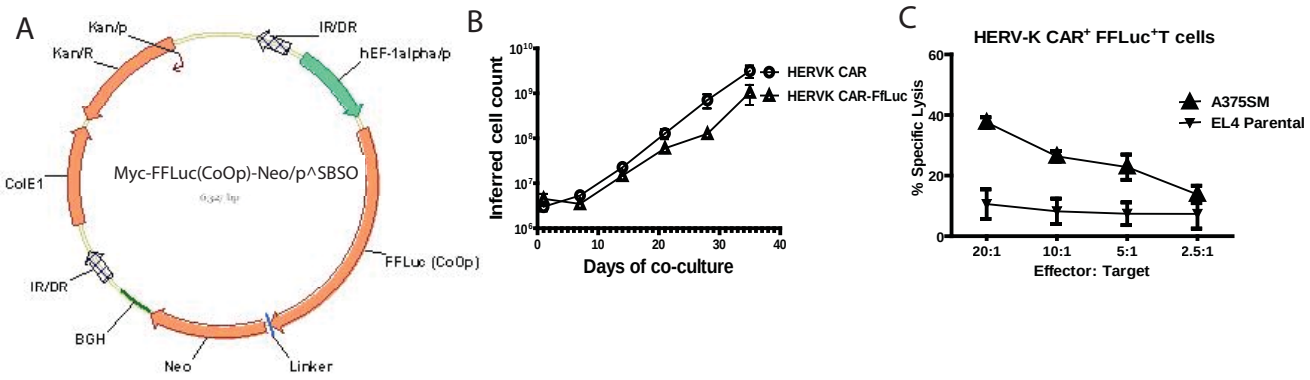
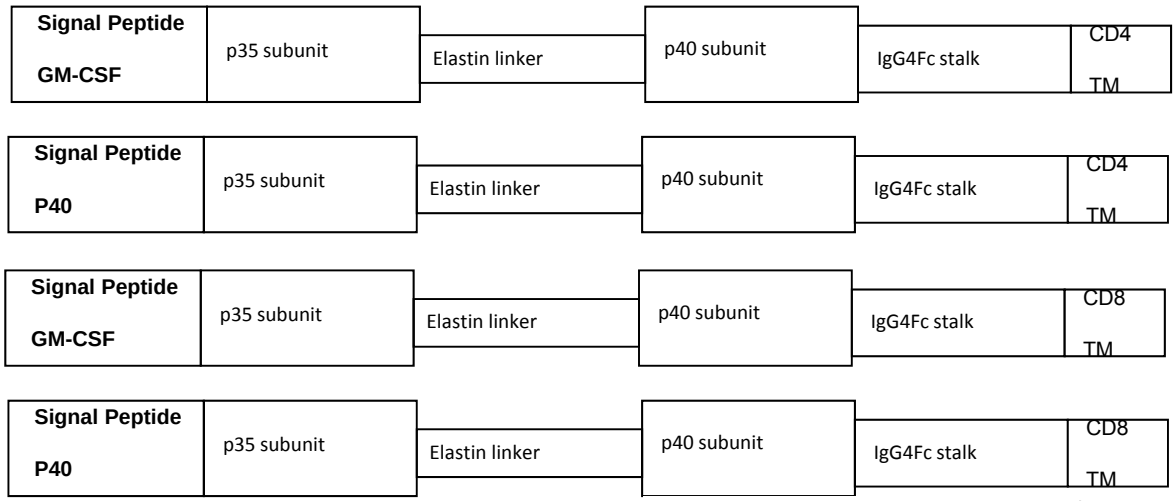
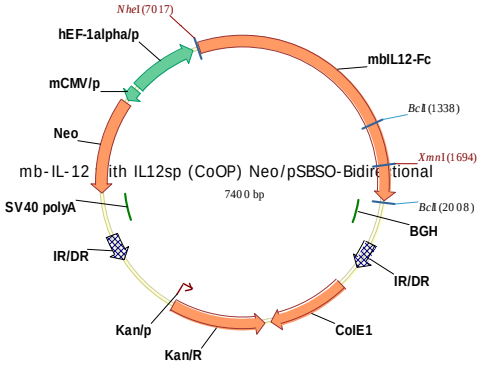


Figure 2: To determine the specificity of HERV-K-specific CAR+T cells, (D) Flow plot of EL4 cells artifically expressing HERV-K antigen was plotted along with HERV-Kneg EL4 parental. (E) A four hour CRA showed a significantincrease (p-value<0.001) in killing EL4 cells expressing rhe antigen compared to the parental by theHERV-K-specific CAR+T cells at varying E:T ratio. (F) Immunoblot assay was perfromed to showHERV-K env specific shRNA lmediated knockdown in A888 cells when compared to A888 parent or A888 treated with scrabelled shRNA. Lower panel shows actin protein expression as control.(G) CRA of HERV-K-specific CAR+T cells with the A888 HERVK KD cells , A888 parental (A888P) and A888 scrambelled (scra) showed a significant antigen specific killing by the T cells between the A888 P and A888KD. Alldata represent the mean of twoindependent experiment by 3 donors preformed in triplicates. 2-way ANOVA with bonferroni posttest was used for (E) to compare EL4 parental to HERV-K+ EL4and one way ANOVA with Newman-Keuls multiple comparison test for (F) to compare A888KD to A888 P .



Supplementary Figure 1: (A) SB Plasmid encoding myc-FfLuc with Neomycin resistance gene (B) Total cell growth of HERV-K-specific CAR^{+}T cells and HERV-K-specific CAR-FfLuc^{+}T cells (C) 4hr CRA of A375SM and EL4 parental cells with HERV-K-specific CAR-FfLuc+T cells,





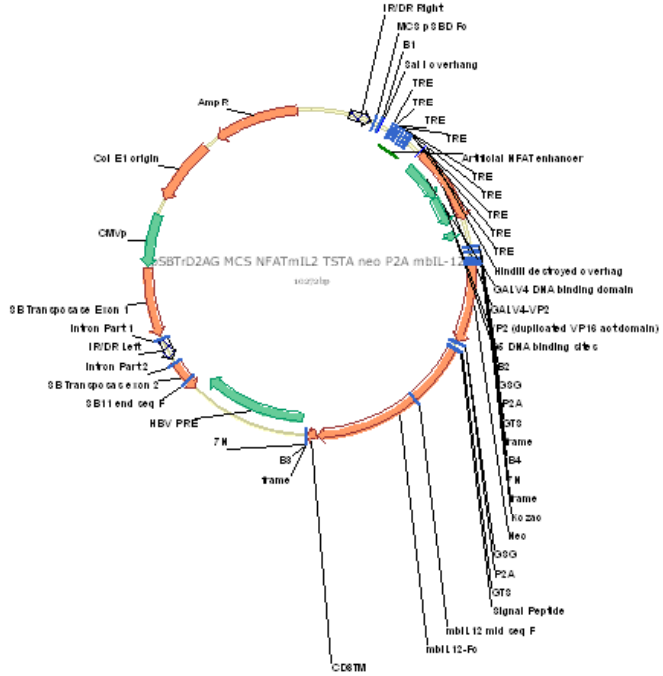


Figure 3A: A representation of various fusion protein of mb-IL-12 with either GM-CSF or p40 as signal peptide and Fc-CD4 or Fc-CD8 as stalk. (B) Bi- directional SB plasmid encoding mb-IL-12 on hEF1a promoter and Neomycin resistance gene on CMV promoter. (C) Unidirectional SB vector with mb-IL-12 under NFAT promoter.

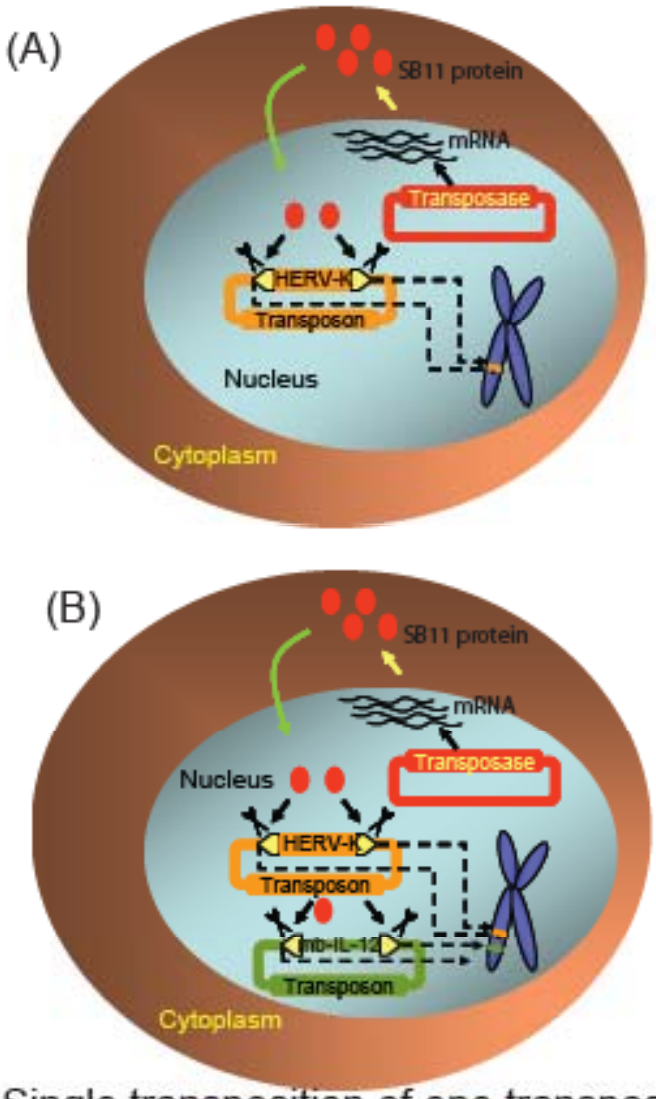


Figure 4: (A) Single transposition of one transposon (HERV-K CAR)
(B) Double transposition of two transposon (HERV-K CAR and mb-IL-12)

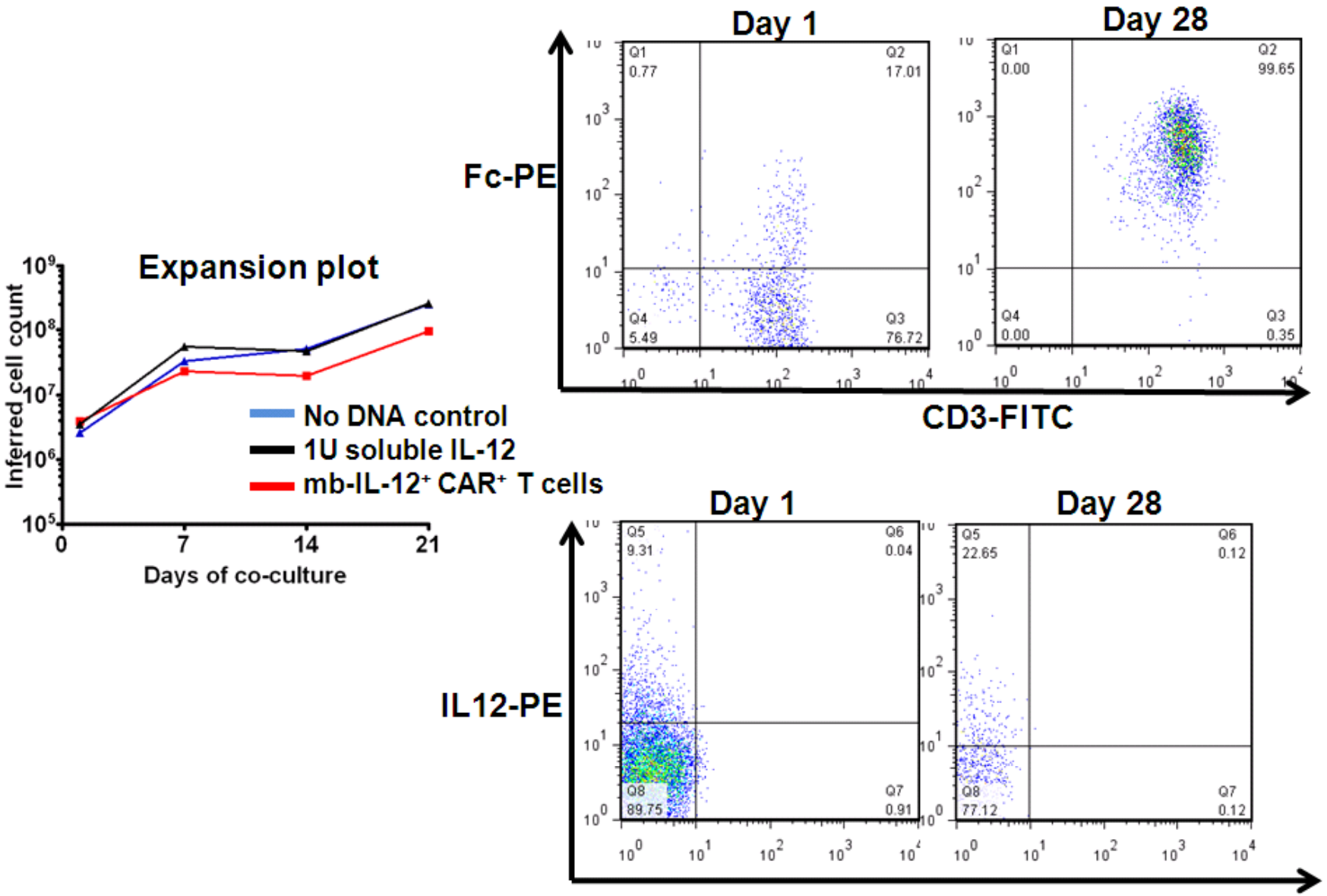


Figure 5 (A) Expansion plot representing the growth of No DNA cells, HERV-K CAR$^+$ T cells with soluble IL-12 and mb-IL-12$^+$CAR$^+$ T cells , : (B) HERV-K CAR expression on Day 1 and 28 on mb-IL-12$^+$CAR$^+$ T cells , (C) IL-12 (p40) expression on Day 1 and 28 on mb-IL-12$^+$CAR$^+$ T cells

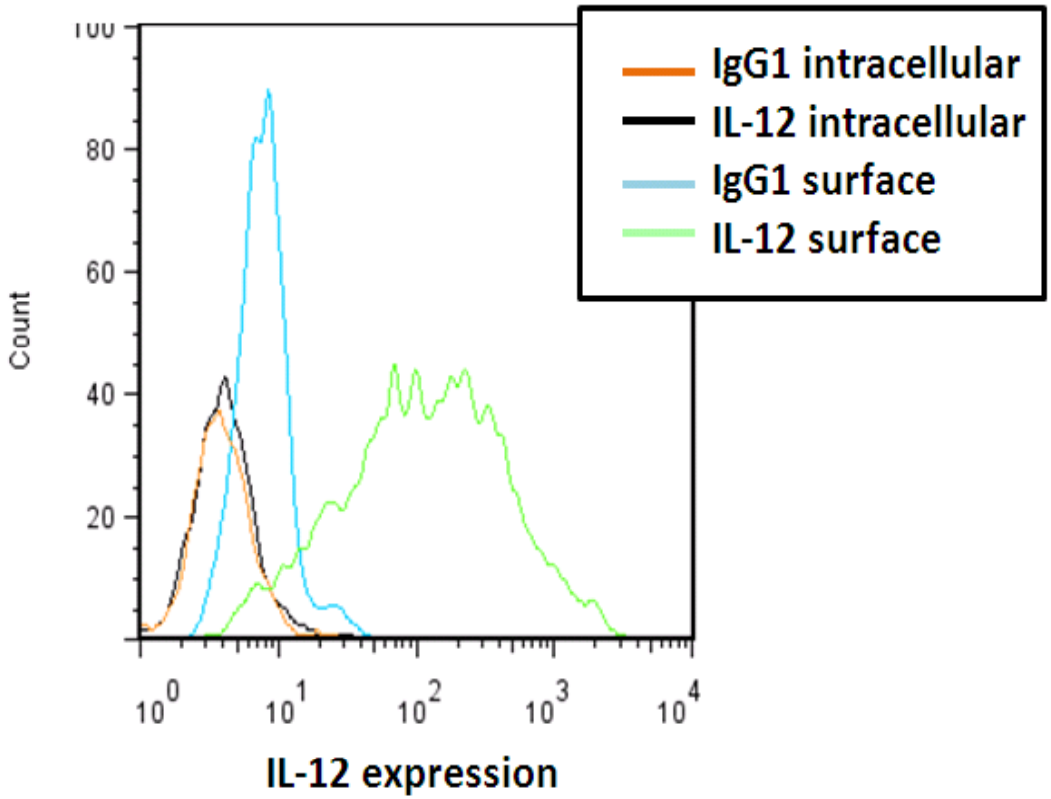


Figure 5D : Plot representing surface and intracellular expression of IL-12 and isotype on mb-IL-12$^+$CAR$^+$T cells.

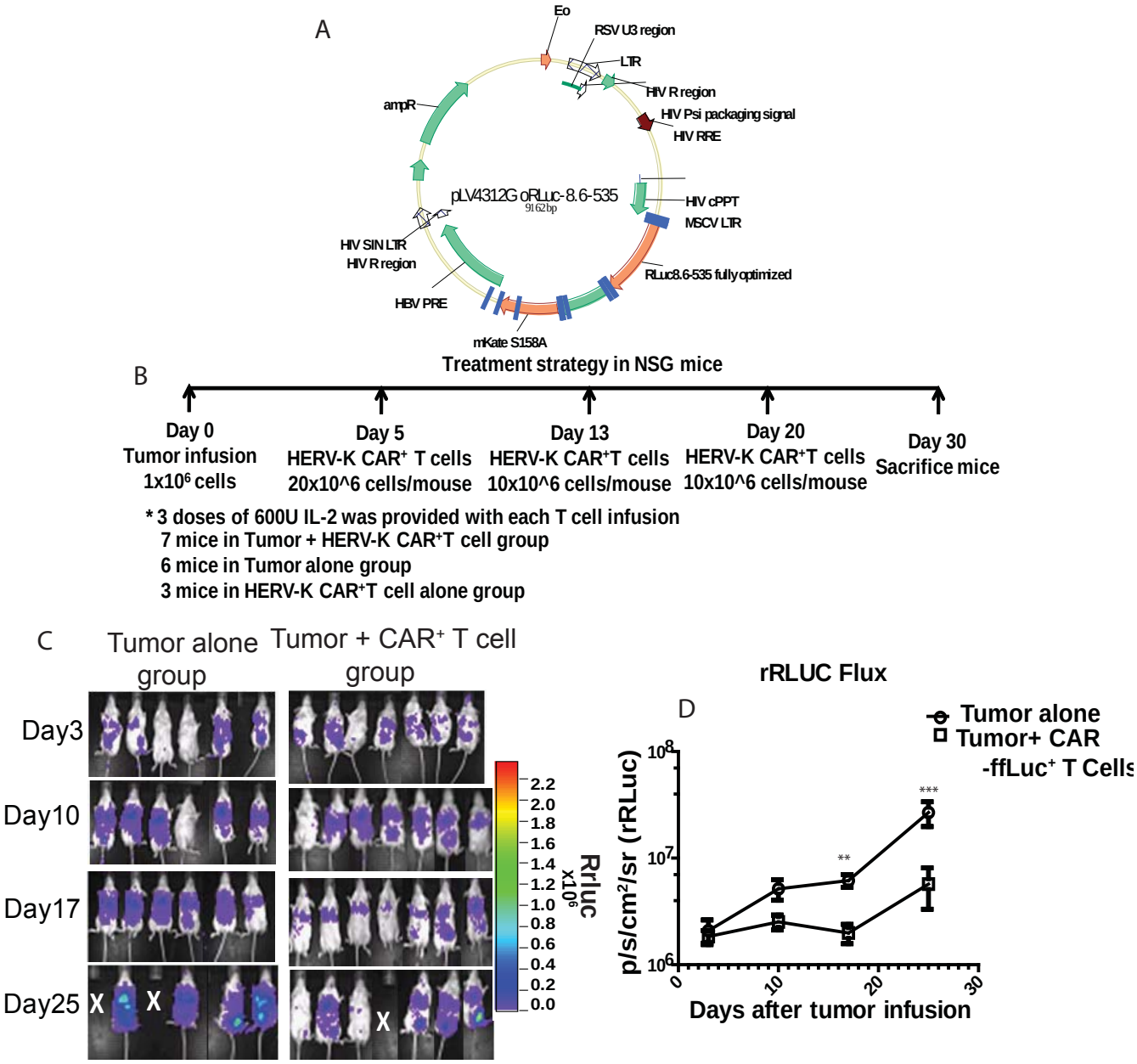


Figure 6: In vivo antitumor activity of HERV-K-specific CAR⁺T cells. (A) Vector encoding rrLuc and mkate (B)Schematic of experiment. (C) Representative images of mice from day 3 to day25. (D) BLI quantification derived from mKate⁺rRLuc⁺HERV-K⁺ A375-SM tumor a **P < 0.01 and ***P < 0.001. ANOVA, analysis of variance; BLI, bioluminescent imaging; CAR, chimeric antigen receptor; ; IL, interleukin;

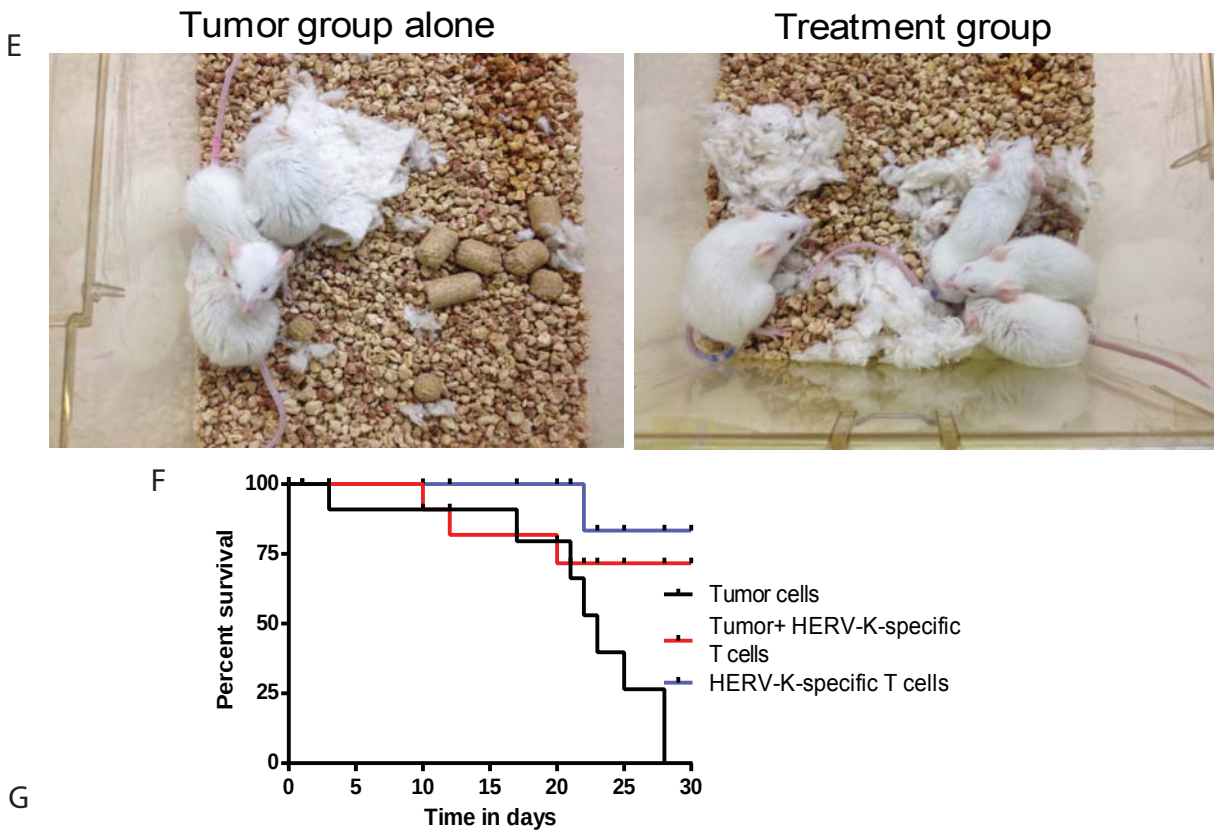


Figure 6(E) Visual difference in health between tumor bearing mice (hunched posture, ruffled fur and weight loss) compared to treatment group (smooth coat and normal weight). (F) Kaplan-mayer survial curve: significant difference in survival with and without treatment (G) Postmortem analysis and quantification of mKate+ve metastatic tumor foci on liver tissues.Statistics performed with Mantel-Cox Test for survival and 2-way ANOVA with Bonferroni's post-test *P<0.1, **P<0.01 and ***P<0.001 (for E).

Janani Krishnamurthy
Houston, TX 77054

jananikrish2005@hotmail.com

GRADUATE RESEARCH ASSISTANT

Immunology ▪ Neurobiology ▪ Targeting Gene Delivery ▪ Animal Imaging ▪ Manage & Mentor

PROFESSIONAL EXPERIENCE

August 2008−Present: Graduate Research Assistant, **Dr. Laurence J.N. Cooper's Lab**, Department of Immunology, MD Anderson Cancer Center, University of Texas, Houston, TX

- Lead scientist on project focused on development of chimeric antigen receptor (CAR) positive T cells targeting an ancient retroviral tumor antigen exclusively expressed on tumor cells and not on normal cells.
- Establish the structural orientation of the ancient retroviral tumor antigen, Human Endogenous Retrovirus-K (HERV-K)
- Assessed the specificity of these CAR$^+$T cells against HERV-K antigen positive tumor cells *in vitro* and *in vivo.*
- Establish bioluminescent metastatic cancer models in mouse.
- Developed cell lines (mouse T cell lymphoma) with artificially forced HERV-K antigen expression.
- Made tumor cell lines negative for HERV-K expression using si-RNA knockdown.
- Successfully expressed membrane-bound interleukine-12 on T cell surface.
- Organize and coordinate research experiments that required involvement of personnel from other laboratories and core facilities to ensure laboratory objectives achieved.
- Research, create and prepare data for publications, presentations, and grant applications.

April 2006−May 2008: Graduate Research Assistant, **Dr. Judy Teale's Lab,** Department of Immunology, University of Texas, San Antonio, TX

- Lead project focused on evaluation of the role of doxycycline in murine model of neurocysticercosis (NCC) infection resulted in a manuscript publication and thesis work.
- Established murine NCC model using intra-cranial parasitic infection of *Mesocestoid Corti.*
- Developed a Q-PCR based assay for rapid quantitative detection of Th1 cyotkine transcripts during NCC in murine brain.
- Managed and maintained cryostat, tissue slicing for immunohistochemistry and immunoflouresence studies.
- Managed Leica deconvolution microscope and characterized the cellular infiltration and involvement of extracellular matrix components in murine brain during NCC.
- Researched, created and prepared data for publications, presentations, and grant applications.

April 2006−May 2008: Graduate Teaching Assistant, Department of Biology, University of Texas, San Antonio, TX

- Mentored 3 credit hour biology laboratory for freshman year.
- Managed and developed unique assays to help understand the basic concepts of anatomy, molecular, cellular biology and behavioral sciences.
- Prepared tests and managed grading the test papers.

August 2004−May 2005: Undergraduate, Department of Pharmacy, Ramachandra Medical College and Research Institute, Chennai, India.

- CLINICAL PHARMACY: Team counselor involved in educating and obtaining data from hypertensive, diabetic and asthmatic patients at Ramachandra Medical Hospital that resulted in undergraduate thesis work.
- Educated the patient population on dietary habits and obtained data on the medication and dietary habits.
- RESEARCH: Collaborated on an inter-institutional project focused on assessing the antinociceptive effects of herbal drug *Coscinium fenestratum* (gaertn) on mouse induced with pain using formalin which resulted in a manuscript publication.

October 2004−December 2004: Internship, Goldstein Pharmaceuticals, Chennai, India.

- Key player on projects focused on capsule manufacturing and tablet coating technology.
- Collaborated with the regulatory affairs team in documentation of release criteria of a drug.

EDUCATION

University of Texas MD Anderson Cancer Center **Houston, TX**
Ph.D. Student, Cancer Immunology,
2008-current

University of Texas at San Antonio **San Antonio, TX**
Master in Biotechnology, GPA -4.0/4.0
2005

Sri Ramachandra Medical College and Research Institute **Chennai ,TN, India**
Bachelor of Pharmacy , GPA- 4.5/5
2001

GRANTS AND AWARDS

Department of Defense, Breast Cancer Research Program, Pre-doctoral Grant for Targeting an ancient retrovirus using adoptive T-cell therapy for treating breast cancer — August 2010-December 2013

Wei Yu Family Endowed Scholarship in the field of Virology — Feb 2013

Joanna M. Foundation Research Scholarship for Melanoma — March 2013

University of Texas, MD Anderson Cancer Center, Department of Immunology Travel award — May 2012

University of Texas at San Antonio, Presidential award for outstanding academic performance — 2007-2008

Texas grant for international students — 2005-2007

LABORATORY SKILLS

ANIMAL WORK AND IMAGING
Non-invasive bioluminescence and fluorescence imaging, establishment of subcutaneous, intraperitoneal and intravenous tumor xenografts in mice, mice immunization, small surgical operations, development of disseminated metastatic tumor models, intravenous administration of cells and substrates, perfusion, tissue collection, processing, and analysis, immunochistochemistry.

CELL CULTURE
Propagation, maintenance, and cryopreservation of various cell lines, isolation and propagation of primary T cells and T cell subsets such as regulatory T cells (T regs), primary tumor and cell cultures, transfection, generation of the stable cell lines, cell enrichment and evaluation, flow cytometry (FACS), reporter gene assays, cell proliferation assays.

T cell specificity tests including Chromium release assay, Caspase assay and IFN-γ cytokine release assay, T-reg suppression assay.

MOLECULAR BIOLOGY
Molecular cloning (traditional restriction-ligation-based, PCR-based, and by homologous DNA recombination in *E. coli*), construction of recombinant lentiviral genomes, PCR, qRT-PCR (TaqMan), RNA isolation, DNA sequence analysis, spectroscopy (UV/Vis, fluorescence).

VIROLOGY
Propagation and purification of recombinant lentiviral vectors, viral transduction, virus titration, cytotoxicity and gene transfer assays.

PROTEIN EXPRESSION, PURIFICATION AND ANALYSIS
Protein expression in bacteria and mammalian cells, protein purification by chromatography, immunoprecipitation, Western blotting, ELISA and Flow cytometry.

MICROSCOPY
Phase contrast, immunofluorescence staining of cultured cells, quantitative analysis of IFC data. Bio-station time lapse imaging for analyzing T cells and tumor cell interaction in vitro over 24 hr period of time. Whole body immunoflourescence imaging -Leica Steromicroscope.

COMPUTER SKILLS

Windows, MS Word, Excel, Power Point, Adobe Photoshop, Adobe Acrobat, Adobe Illustrator, EndNote, Vector NTI, Living Image, Primer Express, IP Lab and Image J.

ORAL PRESENTATIONS

1. T-cell therapy targeting an ancient retrovirus during melanoma, American Society for Gene and Cellular Therapy, Philadelphia, May 2012

2. T-cell therapy targeting an ancient retrovirus during breast cancer, Breast Cancer Research- Era of Hope, Department Of Defense, Florida, May 2011

3. General methods of standardization of herbal drugs, the 55th Indian Pharmaceutical Congress, Chennai, India, Dec 2003.

PEER-REVIEWED RESEARCH PUBLICATIONS

1. Doxycycline treatment decreases morbidity and mortality of murine neurocysticercosis: evidence for reduction of apoptosis and matrix metalloproteinase activity. Alvarez JI, **Krishnamurthy J**, Teale JM. **Am J Pathol 2009** Aug; 175(2):685-95.

2. Antinociceptive Effects of *Coscinium fenestratum* (gaertn) on mouse formalin test. **International Journal Biomedical Research. 2004** 15(1):73-75.

SELECTED ABSTRACTS

1. **Krishnamurthy J**, Olivares S, Rycaj K, Plummer JB, Maiti S, Ling Z, Singh H, Huls H, Wang-Johanning F, Cooper LJ. Targeting an ancient retrovirus expressed in melanoma using adoptive T-cell therapy. American Society for Gene and Cellular Therapy (ASGCT), Philadelphia, May 2012.

2. Krishnamurthy J, Olivares S, Maiti S, Ling Z, Singh H, Huls H, Wang-Johanning F, Cooper LJ. Targeting an ancient retrovirus expressed in melanoma cells using adoptive T-cell therapy. Breast Cancer Research- Era of Hope by Department Of Defence, Florida, May 2011.